Remarks

This amendment is made to conform the application with the provisions of 37 CFR §§1.821 through 1.825. I hereby certify that no new material is being added by this submission.

The Commissioner is hereby authorized to charge to Deposit Account 19-0065 any fees under 37.CFR 1.16 or 1.17 as required by this paper.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this amendment, or if the Examiner believes a telephone interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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JL/srp
Attachments: Submission of Sequence Listing and Statement Under §1.821; New sequence pages 1-2; copy of Notice from PTO.

Marked-Up Paragraphs

Page 8, lines 1-6:

The term "fusion protein" denotes the covalent attachment of two or more proteins whereby at least one biological activity of each protein is retained when the fusion protein is expressed. Thus, in a preferred embodiment, sCD40 is expressed as a fusion protein with GM-CSF, in which a linker polypeptide is encoded within the vector in such a manner as to encode an in-frame polypeptide that connects the two proteins. A suitable linker polypeptide is that encoded by the sequence 5'-GCCGCCGCCGCC-3' (SEQ ID NO: 1).

Page 14, lines 2-18:

The cDNA encoding the murine membrane-bound CD40 is obtained by reverse transcriptase-polymerase chain reaction (RT-PCR) of total RNA prepared from spleens of Balb/c mice. Extraction of RNA and RT-PCR is performed as described. A pair of primers is synthesized according to the published sequences and used for amplication of mCD40. The forward primer, 5'-GTC GCT AGC GGG CAG TGT GTT ACG TGC AGT (SEQ ID NO: 2), corresponds to nucleotides 68-89, published in the Journal of Immunology vol. 148, 620-626(2) 1992, which corresponds to a site starting immediately after the putative signal peptide of the mature murine CD40 protein. This primer includes the addition of a 5' Nhel restriction enzyme site and a GTC sequence, the GTC allowing more efficient digestion of the Nhel site. The reverse primer, 5'-CTT GCT AGC ACA GAT GAC ATT AGT CTG ACT (SEQ ID NO: 3), corresponds to nucleotides 546-566 of the gene sequence published in the Journal of Immunology vol. 148, 620-626(2) 1992, which corresponds to a site starting immediately before the transmembrane domain of the mature murine CD40 protein. This primer includes the addition of a 5' Nhel restriction enzyme site and a CTT sequence, the CTT allowing more efficient digestion of the Nhel site. The final gene product encodes only the extracellular portion of the mature peptide, and excludes the signal peptide, transmembrane and cytoplasmic domains. (Figure 2)

Page 14, lines 19-28:

Amplification of the mCD40 cDNA is performed and the PCR products are purified on a 1.5% agarose gel and directly cloned into the expression vector p at the Nhel sites. The resulting construct, is fully sequenced and no mismatch to published sequence is found. To construct the sCD40 expression vector, another pair of primers is synthesized. The forward primer 5'-GGGCAGTGTTACGTGCAGT-3' (SEQ ID NO: 4), corresponds to nucleotides 71-90, including a site at the beginning of the primer. Nucleotides 9-70 are predicted to encode the leader sequence. The reverse primer, 5'-ACAGATGACATTAGTCTGACT-3' (SEQ ID NO: 5), corresponds to the nucleotides 545-566. The resulting CD40 cDNA portion (71-566) encoding the entire extracellular domain without the leader sequence is cloned into an expression vector. (Figure 1)